

## Murine *Hox-1.11* homeobox gene structure and expression

(*Hox-1.11* nucleotide sequence/embryonic development)

DONG-PING TAN<sup>\*†</sup>, JACQUELINE FERRANTE<sup>\*</sup>, ADIL NAZARALI<sup>\*</sup>, XIAOPING SHAO<sup>\*</sup>, CHRISTINE A. KOZAK<sup>‡</sup>, VICKY GUO<sup>\*</sup>, AND MARSHALL NIRENBERG<sup>\*</sup>

<sup>\*</sup>Laboratory of Biochemical Genetics, National Heart Lung and Blood Institute, and <sup>‡</sup>Laboratory of Molecular Microbiology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD

Contributed by Marshall Nirenberg, March 31, 1992

**ABSTRACT** The *Hox-1.11* gene encodes a protein 372 amino acid residues long that contains a conserved pentapeptide, a homeodomain, and an acidic region. The amino acid sequence of the homeodomain of *Hox-1.11* is identical to that of *Hox-2.8*, and the N-terminal and C-terminal regions of *Hox-1.11* are similar to those of human HOX2H, which is the equivalent of murine *Hox-2.8*. The *Hox-1.11* gene was shown to reside on murine chromosome 6, which contains the *Hox-1* cluster of homeobox genes. One species of *Hox-1.11* poly(A)<sup>+</sup> RNA approximately 1.7 kb long was detected in mouse embryos, which is most abundant in 12-day-old embryos and progressively decreases during further embryonic development. The most anterior expression of *Hox-1.11* poly(A)<sup>+</sup> RNA in 12- to 14-day-old mouse embryos was shown by *in situ* hybridization to be in the mid and posterior hindbrain. *Hox-1.11* poly(A)<sup>+</sup> RNA also is expressed in the VII and VIII cranial ganglia, spinal cord, spinal ganglia, larynx, lungs, vertebrae, sternum, and intestine.

Mouse chromosomes contain four clusters of homeobox genes that are thought to have originated during evolution by successive duplications of an ancestral Antennapedia–Ultrabithorax (*Antp-Ubx*) cluster of homeobox genes (1, 2). Both the amino acid sequences of the homeodomains derived from these genes and the order of the genes within each cluster have been conserved during evolution (1, 2). The order of the homeobox genes in a mammalian *Antp-Ubx* chromosomal cluster of genes is related to the most anterior site of expression of each gene in the embryo, which is successively displaced toward the posterior, starting with the second gene from the 3' end of the cluster and progressing toward the gene at the 5' end of the cluster (for a recent review, see ref. 3). However, the expression of many of the homeobox genes overlaps toward the posterior. Some of the *Drosophila* homeobox genes in the *Antp* and *Ubx* clusters of genes (4) function as homeotic selector genes (5), which determine unique parts of the body. Homeotic selector genes also may be determinants of cell compartments—i.e., they may regulate genes that encode molecules that enable cells to mix only with cells in the same compartment.

Relatively little is known about the functions of *Antp-Ubx* clusters of homeobox genes in mammals. However, recent evidence suggests that a segmental pattern of rhombomeres is generated during the development of the vertebrate hindbrain. Motorneuron nuclei of branchiomotor nerves V, VII, and IX are produced by rhombomeres 2 and 3, 4 and 5, and 6 and 7, respectively. Furthermore, pairs of hindbrain segments match adjacent branchial arches (6). In addition, cells in the hindbrain of the developing chick embryo do not cross rhombomere boundaries (7), which suggests that rhombomeres correspond to cell compartments in the developing

hindbrain. Krumlauf and Boncinelli and their colleagues (8) have shown that anterior expression boundaries of some *Hox* genes correspond to rhombomere boundaries and have suggested that combinatorial sets of homeobox and other proteins that regulate genes may impart unique positional addresses to hindbrain rhombomeres and associated structures in the branchial region of the embryo.

In this report we describe the nucleotide sequence<sup>§</sup> and the expression of the murine *Hox-1.11* gene during embryonic development.

### METHODS AND MATERIALS

***Hox-1.11* Clones.** Part of the homeobox region of the murine *Hox-1.11* gene (9) (nucleotide residues 1539–1658 in Fig. 2) was used as a template for the synthesis of a <sup>32</sup>P-labeled RNA probe ( $\approx 1.4 \times 10^9$  cpm per  $\mu$ g of RNA), which was used to screen an ICR Swiss mouse genomic DNA library in  $\lambda$ Gem-11 (Promega) for the *Hox-1.11* gene. Hybridization was performed at 65°C in 1 M NaCl/50 mM Tris-HCl, pH 7.6/1% SDS containing 100  $\mu$ g of yeast tRNA per ml and  $3.3\text{--}4.4 \times 10^5$  cpm at <sup>32</sup>P-labeled RNA per ml. Filters were washed (final wash) in  $0.1 \times$  SSC ( $1 \times$  SSC = 0.15 M NaCl/0.015 M sodium citrate, pH 7) containing 0.1% SDS at 40°C for 30 min.

*Escherichia coli* C600 hfl cells (BNN 102) infected with an 11.5-day-old Swiss mouse embryo cDNA library in  $\lambda$ gt10 (Clontech) were plated, and  $2 \times 10^6$  plaques were screened with an <sup>35</sup>S-labeled RNA probe (specific activity,  $1.9 \times 10^9$  cpm per  $\mu$ g of RNA) transcribed from a *Hox-1.11* genomic DNA subclone (nucleotide residues 1494–2220 in Fig. 2). Hybridization was performed in 50% formamide containing 5 $\times$  Denhardt's solution ( $1 \times = 0.02\%$  polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin), 0.5% SDS, 175  $\mu$ g of yeast tRNA per ml, and  $4 \times 10^5$  cpm of <sup>35</sup>S-labeled RNA per ml at 42°C for 20 hr. The final wash was with  $0.1 \times$  SSC/0.1% SDS at 40°C for 1 hr.

**DNA Sequencing.** Genomic DNA and cDNA fragments were subcloned into pBluescript II KS(+) (Stratagene). The exonuclease III/mung bean nuclease unidirectional deletion method (10) was used to generate genomic DNA or cDNA subclones with overlapping deletions. Both strands of DNA were sequenced by the dideoxynucleotide chain-termination method (11) using Sequenase 2.0 (United States Biochemical) or *Taq* polymerase DNA sequencing kits and with M13 forward or reverse primers or specific primers. 7-Deaza-dGTP or dITP (United States Biochemical) were used to resolve compressions. An Applied Biosystems DNA sequencer and fluorescent primers or dideoxynucleotides also were used to

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

<sup>†</sup>To whom reprint requests should be addressed at: Laboratory of Biochemical Genetics, National Heart Lung and Blood Institute, National Institutes of Health, Building 36, Room 1C06, 9000 Rockville Pike, Bethesda, MD 20892.

<sup>§</sup>The nucleotide sequences of *Hox-1.11* genomic DNA and cDNA have been deposited in the GenBank data base (accession nos. M93148 and M93292, respectively).

determine DNA sequences. GCG computer programs were used for sequence analysis. We thank Marvin Shapiro for help in using the DNAdraw program (12) to make Figs. 2 and 3.

**Northern Analysis.** BALB/c mouse embryos 10, 12, 14, 16, or 18 days after fertilization were homogenized in 4 M guanidine thiocyanate/0.1 M Tris chloride. RNA was purified by ultracentrifugation through 5.7 M CsCl/10 mM EDTA (13). Poly(A)<sup>+</sup> RNA was obtained by oligo(dT)-cellulose column chromatography (13) and then was fractionated by gel electrophoresis (1% agarose/formaldehyde gels; 10  $\mu$ g of poly(A)<sup>+</sup> RNA per lane). A 510-base-pair (bp) cDNA fragment starting from the 5' end of the cDNA clone without the homeobox (nucleotide residues 345–821 and 1462–1494 in Fig. 2) was labeled with [<sup>32</sup>P]dCTP (2.1  $\times$  10<sup>9</sup> cpm per  $\mu$ g of DNA) by the random primer method (13). Hybridization was performed at 42°C for 22 hr in 50% formamide containing 5.8  $\times$  10<sup>6</sup> cpm of the <sup>32</sup>P-labeled cDNA probe per ml, 5 $\times$  SSPE (1 $\times$  = 0.18 M NaCl/10 mM phosphate, pH 7.4/1 mM EDTA), 5 $\times$  Denhardt's solution, 0.5% SDS, and 200  $\mu$ g of yeast tRNA per ml. The final wash at 65°C was with 0.1 $\times$  SSC/0.1% SDS.

**Identification of the Chromosome That Contains the *Hox-1.11* Gene.** Seventeen Chinese hamster  $\times$  mouse somatic hybrid cell lines (14) were used to identify the mouse chromosome that contains the *Hox-1.11* gene. Chromosomes from 10 hybrid cell lines were identified by Giemsa-trypsin banding; mouse chromosomes from other cell lines were identified by isozyme analysis. For Southern analysis, 10  $\mu$ g of DNA from each line of hybrid cells was incubated with *Hind*III, subjected to electrophoresis in a 0.4% agarose gel, and then transferred to a nylon membrane. The hybridization solution contained 2.8  $\times$  10<sup>6</sup> cpm of <sup>32</sup>P-labeled cDNA (nucleotide residues 345–821 and 1462–1494 in Fig. 2) per ml, 50% formamide, 6 $\times$  SSPE, 1% SDS, and 133  $\mu$ g of denatured, sheared herring sperm DNA per ml. The final wash of the filters at 60°C was with 0.1 $\times$  SSC/1.5% SDS.

**In Situ Hybridization.** BALB/c mouse embryos 12.5 or 14 days after fertilization were separated from parental tissue and frozen as described by Dony and Gruss (15). Sections 10  $\mu$ m thick were cut in a cryostat at –20°C and collected on slides coated with poly(L-lysine). Sections were fixed and hybridized by modification of the method described by Hogan *et al.* (16). <sup>35</sup>S-labeled RNA probes without the homeobox (1–2  $\times$  10<sup>8</sup> cpm/ $\mu$ g) were prepared from the 5' region of the cDNA (nucleotide residues 429–821 and 1462–1494 in Fig. 2) by incorporation of uridine 5'-[ $\alpha$ -(<sup>35</sup>S)]thio]triphosphate. Slides were washed with 2 $\times$  SSC/1 mM DTT at 50°C, then with 0.2 $\times$  SSC/1 mM DTT at 55°C, and finally with 0.2 $\times$  SSC/1 mM DTT at 60°C (1 hr each wash).

## RESULTS AND DISCUSSION

Two million recombinants from a murine genomic DNA library were screened for the *Hox-1.11* homeobox gene by using <sup>32</sup>P-labeled RNA synthesized from PCR-amplified, cloned mouse genomic DNA corresponding to the *Hox-1.11* homeobox region described previously (9). Three *Hox-1.11* clones,  $\lambda$ T7,  $\lambda$ 16, and  $\lambda$ 33, were obtained. In addition, two million recombinants from an 11.5-day-old mouse embryo cDNA library were screened with a genomic DNA fragment corresponding to most of exon 2 of *Hox-1.11* genomic DNA, and one *Hox-1.11* cDNA clone, 1250 bp long, was obtained. The structure of the *Hox-1.11* gene and partial restriction maps of *Hox-1.11* genomic DNA and cDNA are shown in Fig. 1. The *Hox-1.11* gene contains two exons separated by a small intron.

Four thousand and forty nucleotide residues of *Hox-1.11* genomic DNA and 1250 residues of cDNA were sequenced. The composite nucleotide sequence of *Hox-1.11* genomic DNA and cDNA and the deduced amino acid sequence of the *Hox-1.11* homeobox protein are shown in Fig. 2. The *Hox-*

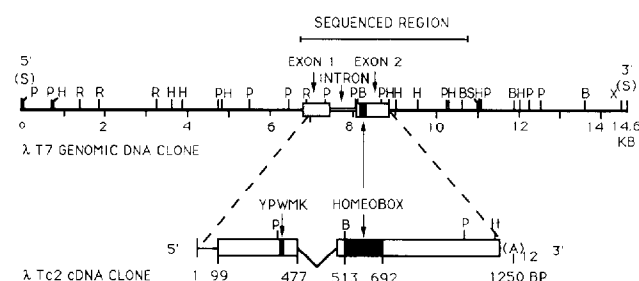


FIG. 1. Partial restriction maps of *Hox-1.11* clones  $\lambda$ T7 genomic DNA in  $\lambda$ Gem-11 and  $\lambda$ Tc2 cDNA in  $\lambda$ gt-10. Exons 1 and 2 in cloned genomic DNA are represented by grey boxes and intron 1 by a narrow open box. The black box in exon 2 of cloned genomic DNA and cDNA corresponds to the homeobox. The location of the 4 kilobases (kb) of genomic DNA that was sequenced is shown above the genomic DNA restriction map. The boxed regions in cDNA represent the coding portion of the cDNA. The black box in exon 1 of the cDNA corresponds to the conserved pentapeptide core, Tyr-Pro-Trp-Met-Lys (YPWMK). Abbreviations for restriction enzymes are as follows: B, *Bam*HI; H, *Hind*III; P, *Pst* I; R, *Eco*RI; S, *Sac* I; X, *Xba* I.

*1.11* gene contains a 1116-nucleotide-residue open reading frame that encodes a protein of 372 amino acid residues with a calculated  $M_r$  of 40,793 and a pI of 5.67. The first ATG in the open reading frame (nucleotide residues 443–445) is assumed to be the initiation codon for protein synthesis; however, only 6 of the 10 nucleotide residues in the putative translation initiation site match the Kozak consensus sequence for initiation of protein synthesis, GCC (A or G) CCATGG (17). The ATG codon is preceded by two adjacent in-frame termination codons 102 nucleotide residues upstream of the ATG. Exon 1 encodes 126 amino acid residues with a conserved hexapeptide core, Glu-Tyr-Pro-Trp-Met-Lys, found in some vertebrate homeobox proteins. Similar sequences also are found in homeotic homeobox proteins of *Drosophila*. Comparison of the nucleotide sequences of *Hox-1.11* genomic DNA and cDNA revealed a 640-bp intron with an unusual 5' splice site, AG  $\downarrow$  GTCAGT. Only 3% of approximately 400 vertebrate 5' splice sites examined contain C as the third nucleotide residue from the 5' terminus of the intron (18); however, the splice branch site and 3' splice site match the consensus sequences perfectly. Exon 2 encodes a homeodomain (amino acid residues 139–198), which is followed by a region with 9 acidic amino acid residues out of 12 residues (amino acid residues 214–225).

Four potential phosphorylation sites for protein kinase C, one for cAMP-dependent protein kinase A, and six for casein kinase II are shown in Fig. 2. Two putative protein kinase C phosphorylation sites are within the homeodomain: Ser-139, the first amino acid residue in the homeodomain, and Thr-179, the amino acid residue immediately before the putative third  $\alpha$ -helix of the homeodomain, which is thought to be the major DNA binding site of the protein. The last amino acid residue in the homeodomain, Thr-198, is a possible phosphorylation site for cAMP-dependent protein kinase A. One putative protein kinase C phosphorylation site is within the acidic domain, and a casein kinase II site, Ser-227, is the second residue after the acidic domain. The possibility that the activity of *Hox-1.11* protein as a regulator of gene expression is reversibly controlled by phosphorylation and dephosphorylation of serine or threonine residues deserves further study.

The termination codon and polyadenylation signal (AT-TAAA) are separated by only four nucleotide residues. AT-TAAA was reported to serve as a polyadenylation signal in 12% of 269 vertebrate cDNAs surveyed, and the polyadenylation activity of AT-TAAA was shown to be 77% of the activity found for AATAAA (19). Comparison of the nucleotide sequences of *Hox-1.11* genomic DNA and cDNA showed

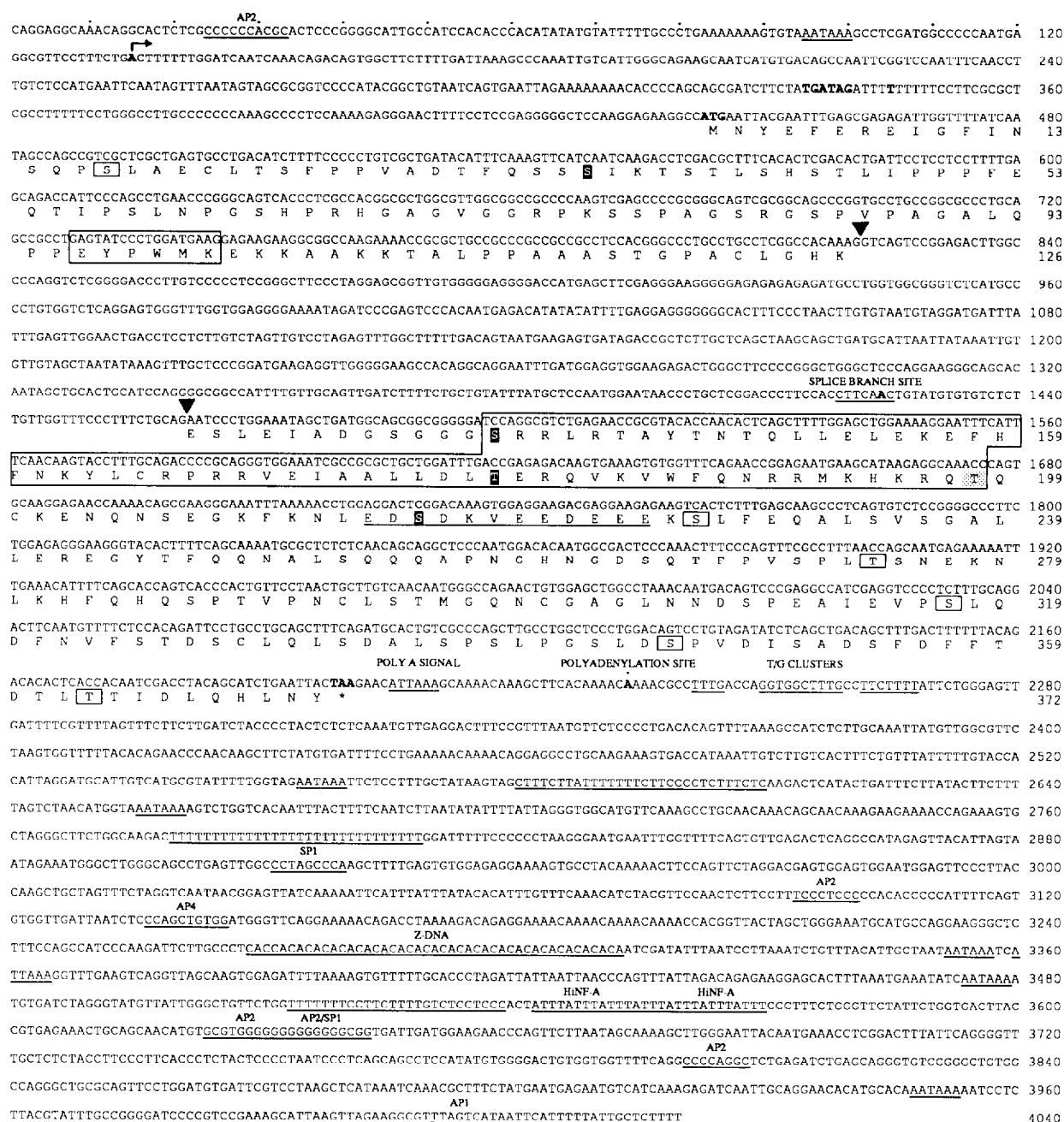


Fig. 2. The nucleotide sequence and the deduced amino acid sequence in single-letter code of the murine *Hox-1.11* gene. The nucleotide sequence is a composite obtained by sequencing 4,040 nucleotide residues of *Hox-1.11* genomic DNA (clones  $\lambda$ 16 and  $\lambda$ 17) and 1,250 residues of clone  $\lambda$ Tc2 cDNA, which correspond to nucleotide residues 345–821 and 1462–2234. Numbers on the right correspond to deoxynucleotide or amino acid residues. The conserved hexapeptide, EYPWMK, and the homeobox are enclosed within boxes. *Hox-1.11* mRNA synthesis is initiated at nucleotide residue 135. The black inverted triangles correspond to RNA splice sites. The splice branch recognition sequence near the end of the intron is underlined, and the branch site is shown as a boldface letter (nucleotide residue 1424). Putative sites for phosphorylation catalyzed by protein kinase C are shown as white S or T residues on black backgrounds; a black T shown on a grey background (T-198) is a putative phosphorylation site catalyzed by cAMP-dependent protein kinase A. S or T residues enclosed within open boxes are possible phosphorylation sites catalyzed by casein kinase II. The polyadenylation signal sequence, ATAAA (2206–2211), is underlined, and the polyadenylation site (nucleotide residue 2234) is shown in boldface letters. T/G clusters on the downstream side of the polyadenylation site and six additional polyadenylation signal sequences are underlined. Some putative binding sites for proteins that regulate gene expression also are underlined. Two polypyrimidine regions and 30 sequential T residues also are underlined (30 T residues were found with  $\lambda$ 16 DNA, 20 with clone  $\lambda$ T7 DNA).

that nucleotide residue 2234 functions as the polyadenylation site. T/G clusters, which may play a role in the formation of poly(A)<sup>+</sup> RNA (for review see ref. 20), were found downstream of the polyadenylation site. Six additional polyadenylation signals were found in the 3' untranslated region of the *Hox-1.11* gene, but we do not know whether they function as alternative polyadenylation signals.

Primer extension experiments (not shown here) revealed one major site for initiation of Hox-1.11 mRNA synthesis at nucleotide residue 135. Further work is needed to determine whether the AATAAA sequence 40 nucleotide residues upstream of the initiation site for mRNA synthesis functions as a TATA box. The first nucleotide residue of  $\lambda$ Tc2 cDNA corresponds to nucleotide residue 345 shown in Fig. 2:

Hox-1.11	MMMEFEREIGFINSQPSLAECLETSFPPVAVLTFFQSSIKKSTLSHSTLIPP	50
HOX2H	MMMEFEREIGFINSQPSLAECLETSFPPVAVLTFFQSSIKKSTLIPP	47
	PFEQTLPSPNPGSHFRHAGVGGRPKSSPAGRGSPVAGALCPPEMFWMEKKRAAKRT	109
	PFEQTLPSPNPGSHFRHAGVGGRPKSSPAGRGSPVAGALCPPEMFWMEKKRAAKRT	107
	ALPFAAASITGPACLGHK.....ESLEIADGSGGGRRRLRTAYTNTOLLELEKEFHENKY	163
	SGATSEFPAAASAVFASGVGSPADGGLSEBAGGGGRRRLRTAYTNTOLLELEKEFHENKY	167
	LCRPRRVEIALLDLTERQVKVWFQNRMRKHKROTCKENONSFGKFN.LEDSKVVEED	222
	LCRPRRVEIALLDLTERQVKVWFQNRMRKHKROTCKENONSFGKFN.LEDSKVVEED	226
	EEKSLFPAALGVSCALLEREGYTFQNALSQQAENGHNDSTTFVSPVLSNEKNLKH	282
	.....EPASPGGPSASRAA..WEACCHPPEVVMGALSADPRPLAV.....R	266
	FQHSPTVNL.....STMGQNCGAGLNDSPEAIEVFSLDENFSTDSCLQLSE	334
	LEGAGASSPSCALRGAGGLEPGPLPDDVFSGRDSE.....FLEDFNFPAADSCQLQSS	320
	ALCPSPGLSDSPVDLSADSDFFETLTLTIDLCHLNY*	372
	GLSPSLGSLDSPVFFSEBELDFFETLTLTIDLCHLNY*	356

FIG. 3. The amino acid sequence in single-letter code of murine Hox-1.11 protein is compared with that of human HOX2H protein (24). The amino acid residues of the conserved hexapeptide and the homeodomain are underlined. White amino acid residues on a black background correspond to identical amino acid residues; black amino acid residues on a grey background represent groups of conservative amino acid replacements, which are as follows: S, T, G, A, P/L, M, I, V/E, D, Q, N/R, K, H/F, Y, W/ and C.

therefore, 211 nucleotide residues are missing from the 5' end of  $\lambda$ Tc2 Hox-1.11 cDNA.

The untranslated regions of the *Hox-1.11* gene contain possible sites for proteins that are known to regulate gene expression, such as AP-1, AP-2, AP-4, SP-1, and HNF-A (seven sequential ATTT direct repeats constitute two HNF-A sites). The 3' untranslated region also contains 30 sequential T residues; two additional polypyrimidine regions; a (CA)<sub>22</sub> repeat, which is expected to assume the conformation of Z-DNA under appropriate conditions; and a region rich in G residues.

The amino acid sequence of the murine Hox-1.11 homeodomain is identical to that of the Hox-2.8 (21), which suggests that both homeobox proteins may bind to the same or similar nucleotide sequences in DNA. Hox-1.3 (22) and Hox-2.1 (23) also have homeodomains with identical amino acid sequences. A comparison of the amino acid sequences of murine Hox-1.11 protein and human HOX2H (24) protein (the equivalent of murine Hox-2.8) is shown in Fig. 3. The sequence of the human, rather than the murine homeobox protein is shown because only the sequence of the homeobox region of murine Hox-2.8 has been reported thus far. The homeodomain of Hox-1.11 and HOX2H is the most highly conserved region of each protein; however, many identical amino acid residues or conservative amino acid replacements are present in the N-terminal region, near the conserved hexapeptide, and in the C-terminal region of each protein. Approximately 50% of the amino acid residues of the murine Hox-1.11 and human HOX2H proteins are identical, and an additional 18.6% are conservative amino acid replacements. These results suggest that the *Hox-1.11* and *Hox-2.8* genes originated by duplication of the same ancestral gene and then gradually diverged by mutation.

Murine homeobox gene clusters *Hox-1*, *Hox-2*, *Hox-3*, and *Hox-4* are located on chromosomes 6, 11, 15, and 2, respectively. The chromosome that contains the *Hox-1.11* gene was identified by Southern analysis of DNA preparations obtained from 17 Chinese hamster  $\times$  mouse somatic hybrid cell lines that contain different sets of identified mouse chromosomes (Table 1). Genomic DNA from each hybrid cell line was incubated with *Hind*III, subjected to gel electrophoresis, transferred to a nylon membrane, and hybridized to a <sup>32</sup>P-labeled Hox-1.11 cDNA fragment. The Hox-1.11 cDNA probe hybridized to a 4 kb murine *Hind*III DNA fragment and a 3-kb hamster DNA fragment. DNA from 10 of the 17 hybrid cell lines examined contained the murine 4-kb fragment of the *Hox-1.11* gene. Correlation of the murine chromosome content of each hybrid cell line with the results of Southern

Table 1. Analysis of concordance between *Hox-1.11* DNA hybridization to mouse DNA from 17 Chinese hamster  $\times$  mouse somatic hybrid cell lines and the mouse chromosomes present in each cell line

Mouse chromosome	No. of hybrids*				% discordance
	+/+	-/-	+/-	-/+	
1	8	5	2	2	24
2	9	2	1	5	35
3	4	4	4	2	43
4	7	5	3	2	29
5	2	6	8	1	53
6	10	7	0	0	0
7	8	1	2	6	47
8	8	5	2	2	24
9	5	5	4	2	38
10	1	6	9	1	59
11	0	7	10	0	59
12	6	1	3	5	53
13	7	5	2	2	25
14	1	6	8	1	56
15	10	0	0	6	38
16	6	6	2	1	20
17	9	1	1	6	42
18	9	4	1	1	13
19	8	4	2	3	29
x	7	3	3	4	41

\*Symbols represent the presence (+/-) or absence (-/-) of *Hox-1.11* DNA probe hybridization to a 4-kb mouse DNA *Hind*III restriction fragment determined by Southern analysis and the presence (+) or absence (-) of the mouse chromosome that contains the *Hox-1.11* gene. The number of discordant observations is the sum of the +/- and -/+ observations. The percent discordance is the number of discordant observations, divided by the total number of observations, multiplied by 100. The results show that the *Hox-1.11* gene resides in mouse chromosome 6.

analysis showed that the *Hox-1.11* gene resides on murine chromosome 6, which suggests that the *Hox-1.11* gene is a member of the *Hox-1* cluster of homeobox genes. The amino acid sequence similarity of Hox-1.11 and Hox-2.8 suggests that *Hox-1.11* is the second gene from the 3' end of the *Hox-1* cluster of genes located between *Hox-1.5* and *Hox-1.6*, and that *Hox-1.11*, like *Hox-2.8*, is a member of the *Drosophila* proboscipedia (*pb*) subfamily of homeobox genes.

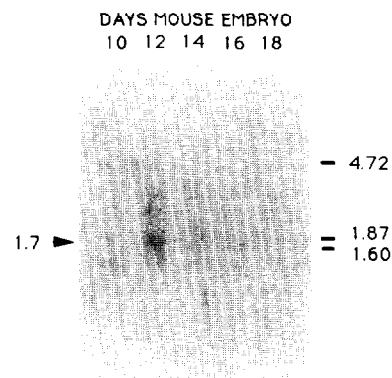


FIG. 4. Northern analysis of Hox-1.11 poly(A)<sup>+</sup> RNA at different stages of mouse embryo development. Each lane contains 10  $\mu$ g of poly(A)<sup>+</sup> RNA from mouse embryos 10, 12, 14, 16, or 18 days after fertilization as indicated. Poly(A)<sup>+</sup> RNA was subjected to electrophoresis, transferred to a Nytran membrane, and hybridized to the 5' portion of Hox-1.11 <sup>32</sup>P-labeled cDNA (without the homeobox). The positions of 28S and 18S ribosomal RNA (4718 and 1874 nucleotide residues, respectively), a 1.6-kb RNA standard, and a diffuse band of Hox-1.11 poly(A)<sup>+</sup> RNA, approximately 1700 residues long are shown.

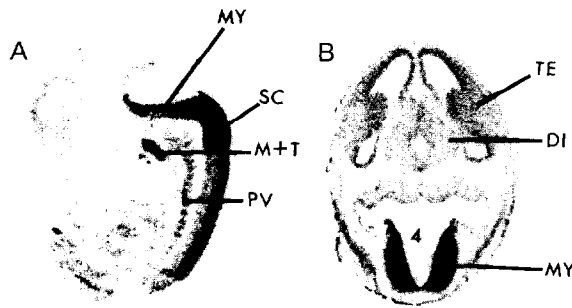


FIG. 5. *In situ* hybridization of a 416-nucleotide-residue Hox-1.11  $^{35}\text{S}$ -labeled RNA probe (without the homeobox) to Hox-1.11 poly(A)<sup>+</sup> RNA in a parasagittal section of a mouse embryo 12.5 days after fertilization (A) and a transverse section of a 14-day-old mouse embryo (B), exposed to x-ray film for 5 and 12 days, respectively. MY, mid and posterior myelencephalon; SC, spinal cord; M, mesenchyme near the larynx; T, thymus; S, sternum; PV, prevertebrae; TE, telencephalon; and DI, diencephalon.

The expression of Hox-1.11 poly(A)<sup>+</sup> RNA during mouse embryo development was determined by Northern analysis of poly(A)<sup>+</sup> RNA from embryos at different stages of development (Fig. 4). The  $^{32}\text{P}$ -labeled Hox-1.11  $\Delta\text{Tc2}$  cDNA probe contained nucleotide residues 1–510 shown in Fig. 1 but not the homeobox. Only one diffuse band of Hox-1.11 poly(A)<sup>+</sup> RNA, approximately 1.7 kb long, was detected. The abundance of Hox-1.11 poly(A)<sup>+</sup> RNA is low in 10-day-old embryos, maximum in 12-day-old embryos, and then progressively decreases in abundance in 14-, 16-, and 18-day-old mouse embryos. The size of Hox-1.11 poly(A)<sup>+</sup> RNA found by Northern analysis (1.7 kb) agrees well with the size of Hox-1.11 mRNA determined by nucleotide sequence analysis [1454 nucleotide residues without a poly(A) tail, and 1704 nucleotide residues assuming a poly(A)<sup>+</sup> tail of 250 residues].

Tissues that contain Hox-1.11 poly(A)<sup>+</sup> RNA in 12.5- and 14-day-old mouse embryos were identified by *in situ* hybridization (Fig. 5). A Hox-1.11  $^{35}\text{S}$ -labeled RNA probe without the homeobox was hybridized to Hox-1.11 poly(A)<sup>+</sup> RNA in sagittal or transverse serial sections of mouse embryos, and sections were subjected to autoradiography. Hox-1.11 poly(A)<sup>+</sup> RNA is expressed prominently in mid and posterior myelencephalon (but not in the pons), spinal cord, larynx, thymus, sternum, and vertebrae. In other sections not shown here, Hox-1.11 poly(A)<sup>+</sup> RNA also was detected in the VII and VIII cranial ganglia, spinal ganglia, lungs, ribs, and intestine. These results agree with and extend a previous report that Hox-1.11 poly(A)<sup>+</sup> RNA in mouse embryos is expressed in posterior hindbrain, cranial ganglia VII and VIII, and mesenchyme of the second and third branchial arches (8).

Nothing is known about the functions of Hox-1.11 protein. However, homologous recombination in embryonic stem cells and transgenic mouse technology have been used to obtain strains of mice with *Hox-1.6* (25, 26) or *Hox-1.5* (27) mutations. Homozygous loss of function mutants of the *Hox-1.6* or *Hox-1.5* genes have different phenotypes, but each phenotype consists of the absence of some tissues and anatomical defects in other tissues in the head and thorax.

Therefore, Hox-1.6 and Hox-1.5 proteins are required for the development of different parts of the head and thorax. *Hox-1.6*, *Hox-1.11*, and *Hox-1.5* genes are the first, second, and third genes, respectively, from the 3' end of the *Hox-1* cluster of homeobox genes and are expressed in different, overlapping patterns in mouse embryos. Therefore, we expect that Hox-1.11 homeobox protein also may be required for the development of some tissues in the head and thorax, such as the medulla oblongata, some cranial ganglia or cranial nerves, and tissues that originate from or interact with the cephalic neural crest.

1. Duboule, D. & Dolle, P. (1989) *EMBO J.* **8**, 1497–1505.
2. Graham, A., Papalopulu, N. & Krumlauf, R. (1989) *Cell* **57**, 367–378.
3. McGinnis, W. & Krumlauf, R. (1992) *Cell* **68**, 283–302.
4. Lewis, E. B. (1978) *Nature (London)* **276**, 565–570.
5. Garcia-Bellido, A. (1977) *Am. J. Zool.* **17**, 613–629.
6. Lumsden, A. & Keynes, R. (1989) *Nature (London)* **337**, 424–428.
7. Fraser, S., Keynes, R. & Lumsden, A. (1990) *Nature (London)* **344**, 431–435.
8. Hunt, P., Gulisano, M., Cook, M., Sham, M.-H., Faiella, A., Wilkinson, D., Boncinelli, E. & Krumlauf, R. (1991) *Nature (London)* **353**, 861–864.
9. Nazari, A., Kim, Y. & Nirenberg, M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 2883–2887.
10. Guo, L. H., Yang, R. C. A. & Wu, R. (1983) *Nucleic Acids Res.* **11**, 5521–5540.
11. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
12. Shapiro, M. (1990) *Binary* **2**, 187–190.
13. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), 2nd Ed.
14. Hoggan, M. D., Helden, N. F., Buckler, C. E. & Kozak, C. A. (1988) *J. Virol.* **62**, 1055–1056.
15. Dony, C. & Gruss, P. (1987) *EMBO J.* **6**, 2965–2975.
16. Hogan, B., Costantini, F. & Lacy, E. (1986) in *Manipulating the Mouse Embryo* (Cold Spring Harbor Lab., Cold Spring Harbor, NY), pp. 228–232.
17. Kozak, M. (1987) *Nucleic Acids Res.* **15**, 8125–8148.
18. Padgett, R. A., Grabowski, P. J., Konarska, M. M., Seiler, S. & Sharp, P. A. (1986) *Annu. Rev. Biochem.* **55**, 1119–1150.
19. Sheets, M. D., Ogg, S. C. & Wickens, M. P. (1990) *Nucleic Acids Res.* **18**, 5799–5805.
20. Birnstiel, M. L., Busslinger, M. & Strub, K. (1985) *Cell* **41**, 349–359.
21. Rubock, M. J., Larin, Z., Cook, M., Papalopulu, N., Krumlauf, R. & Lehrach, H. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 4751–4755.
22. Odenwald, W. F., Taylor, C. F., Palmer-Hill, F. J., Friedrich, V., Jr., Tani, M. & Lazzarini, R. A. (1987) *Genes Dev.* **1**, 482–496.
23. Krumlauf, R., Holland, P. W. H., McVey, J. H. & Hogan, B. L. M. (1987) *Development* **99**, 603–617.
24. Acampora, D., D'Esposito, M., Faiella, A., Pannese, M., Migliaccio, E., Morelli, F., Stornaiuolo, A., Nigro, V., Simeone, A. & Boncinelli, E. (1989) *Nucleic Acids Res.* **17**, 10385–10402.
25. Lufkin, T., Dierich, A., LeMeur, M., Mark, M. & Chambon, P. (1991) *Cell* **66**, 1105–1119.
26. Chisaka, O., Musci, T. S. & Capecchi, M. R. (1992) *Nature (London)* **355**, 516–520.
27. Chisaka, O. & Capecchi, M. R. (1991) *Nature (London)* **350**, 473–479.